The value of urine samples from men with non-gonococcal urethritis for the detection of *Chlamydia trachomatis*

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Abstract

***Chlamydia trachomatis* was sought at first and subsequent clinic visits in urethral swabs and urines from 112 heterosexual men with acute non-gonococcal urethritis (NGU). In comparison with a urethral swab tested by MicroTrak (MT), a urine deposit tested in the same way was 90% as sensitive. Examining a urine deposit by the enzyme immunoassay IDEIA was a little less sensitive (89%) than examining a similar deposit by MT, and was less sensitive (82%) than examining a urethral swab by MT. The results of testing urines were little influenced by collecting them either before or after swabbing the urethra, and there was evidence that examining all of a urine sample by IDEIA would have increased sensitivity. Overall, 55 (49%) of the men were diagnosed as *C trachomatis*-positive based on the results of testing both a urethral swab and a urine sample. Furthermore, small numbers of chlamydiae were detected by examining urine by MT and, to a lesser extent, by IDEIA, so that there is no reason why this non-invasive approach should not be successful in men other than those with acute NGU.**

Introduction

Swabbing the urethra has been the traditional approach to collecting specimens for the detection of *Chlamydia trachomatis* in men with non-gonococcal urethritis (NGU). Numerous observations have been made on the efficacy of various methods, for example culture, direct immunofluorescence and enzyme immunoassays, for detecting chlamydiae in such specimens.[1] By these means, chlamydiae have been detected in up to half of heterosexual men with NGU. In our hands, the MicroTrak (MT) direct immunofluorescence technique (Syva, UK) was as sensitive as culture[2] and we have shown recently that the polymerase chain reaction (PCR) is as sensitive as the MT technique.[3] Two enzyme immunoassays (Chlamydiazyme and IDEIA) were less sensitive than culture or MT.[4–6] However, pooling two swabs from the cervix and, thereby, increasing the concentration of chlamydial antigen enhanced the sensitivity of IDEIA to an acceptable level.[6]

Examination of urine from men with urethritis by a cell culture technique has not been regarded as a sensitive way of detecting *C trachomatis*.[7] However, suggestions have been made recently that examination of urine might still provide a sensitive non-invasive approach to detecting *C trachomatis* if this were done by IDEIA[8] and the results of some comparative tests, albeit on a small number of chlamydia-positive patients, have been encouraging.[9] We have, therefore, also examined the possibility of using urine. Our approach has been to take urethral swabbing by MT as the "gold" standard and compare urine tested by MT against it in order to determine the value of urine as a sample. We then compared the value of IDEIA with that of MT for testing urine. The PCR was used as an additional test when the results of the other two tests were discordant. Finally, we compared the procedure most likely to be used in practice, namely urine tested by IDEIA, with the "gold" standard.

Materials and methods

Patients

Heterosexual men attending the Jefferiss Wing (St Mary's Hospital) with symptoms and signs of acute NGU were studied. The latter was diagnosed if there were $\geqslant 5$ polymorphonuclear leucocytes per high power field ($\times$ 800) in a Gram-stained smear of urethral discharge, and gonorrhoea was excluded by microscopy and culture. Men who had taken antibiotics known to be active against *C trachomatis* in the previous three months were excluded. Ethical Committee approval for the study was obtained prior to commencement and informed consent to take specimens was sought.

Procedure

A nasopharyngeal swab (MW 142; Medical Wire and Equipment Co., Corsham, Wilts) was passed 2–4 cm

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into the urethra and rolled on a MicroTrak slide. It was then agitated in 400 μl of distilled water to provide a specimen for the PCR. A second swab was passed 4–6 cm into the urethra for a second PCR specimen. The first 15–20 ml of voided urine (first pass urine; FPU) was then obtained. For some patients, 15–20 ml of FPU were obtained before the first swab. The Jefferiss Wing is a "walk-in" clinic, so there was variation in the length of time for which subjects had held their urine prior to producing specimens, from 10 min to overnight.

Patients who did not have severe symptoms were asked to collect an early morning specimen of urine (EMU) the following day, before commencing treatment, and deliver it to the clinic during the morning. All patients were asked to re-attend after one and two weeks for repeat tests.

Handling of specimens

Samples of urine were stored at 4°C for a maximum of three days. They were warmed at 37°C to dissolve any deposit which formed on cooling and "whirlimixed" to break up threads and to distribute the cell content evenly. Each urine sample was then divided into three aliquots which were centrifuged at 3,000 rpm for 30 min in a MSE Mistral 2000 centrifuge. The deposits were used as follows: one was resuspended in 100 μl of distilled water, and 10μl of this were dried on a MicroTrak slide and fixed in acetone; one was resuspended in 1 ml of IDEIA transport buffer and stored at − 70°C until tested: the third deposit was resuspended in 400 μl of distilled water and used for the PCR.

Techniques for detecting C trachomatis

Direct immunofluorescence. The MicroTrak (MT) test (Syva, UK) was used as described previously.[2] Urethral smears and urine deposits were fixed in acetone and stained with 15 μl of MT direct specimen reagent. Positive smears were graded as ± (1–10EBs/smear), + (11–100EBs/smear), + + (101–1000EBs/smear) or + + + (> 1000 EBs/smear). Specimens were considered inadequate if they contained few epithelial cells in the absence of EBs, or if the smear was too thick for individual cells to be brought into focus on microscopy.

Enzyme immunoassay. The IDEIA (Novo Nordisk Diagnostics, UK) procedure was undertaken according to the manufacturer's instructions and as described previously.[6]

Polymerase chain reaction (PCR). The PCR was performed as described elsewhere.[3] Briefly, DNA from specimens was amplified in 100 μl PCR reaction buffer containing 1 μg of each primer (HP1 and HP2), 2 mM $MgCl_2$, 50 mM KCl, 10 mM Tris-HCl pH8·4, 0·01% gelatin, 0·05% Tween-20 (Sigma), 0·2 mM dNTPs (Pharmacia) and 2·5 units Amplitaq DNA polymerase (Perkin Elmer Cetus). The conditions for the reaction were as follows: 45°C for 1·5 min, 72°C for 3·0 min and 94°C for 1·5 min for 35 cycles with the temperature maintained at 72°C for 9·9 min during the final cycle. 2 μl of the reaction product were amplified a second time replacing primer HP1 with HP3.

Results

Prevalence of C trachomatis at first clinic visit

Urethral smears from 48 (43%) of 112 men at their first clinic attendance were positive by MT.

Comparison of testing urethral smears by MT with urine deposits by MT

Of 192 pairs of urethral and FPU specimens collected at the first and subsequent clinic visits from 112 men, the results were in concordance for 182 of them (table 1), the sensitivity and specificity of using urine compared to a urethral swab being 90% and 97%, respectively. The positive predictive value (PPV) was 90% and the negative predictive value (NPV) was 97%. Details of the discordant results are shown in table 2. Of five patients who were positive only in the urethra by MT, three had these results supported by the PCR test; the results of the MT tests indicated that only very few organisms were available in the urethra for transfer to the urine, or that the urine samples were inadequate for the assay. Similarly, of

Table 1 Results of testing urethral smears by MT compared with urine deposits by MT

		No. of urine samples that were		
		Positive	*Negative*	*Total*
No. of urethral smears that were	Positive	44	5	49
	Negative	5	138	143
	Total	49	143	192

Table 2 Details of discordant results obtained when testing urethral and urine samples by MT

Specimen no.	*Result of test on*				
	Urethral smear by		*Urine sample by*		
	*MT**	*PCR*	*MT**	*PCR*	*IDEIA*
1	≤10	+	−	−	−
2	≤10	+	−	−	−
3	≤10	−	IN†	−	−
4	>100	+	IN	−	−
5	≤10	−	−	−	+
6	IN†	−	11–100	−	+
7	IN	+	≤10	+	+
8	−	+	≤10	+	−
9	−	−	≤10	−	−
10	−	+	11–100	−	+

*No. of EBs; †inadequate specimen; ‡not done.

five patients whose urine samples only were positive, two had urethral specimens that were inadequate by MT and a further two patients had urine samples that contained fewer than 10 EBs, indicative of a low level of infection in the urethra. However, three of the latter patients had urethral specimens that were positive by the PCR.

Comparison of testing urine deposits by MT with urine deposits by IDEIA

The results of staining by MT 192 FPU and 38 EMU samples obtained at the first and subsequent clinic visits were compared with those of testing an aliquot of the same samples by IDEIA (table 3). There was concordance for 218 samples, the sensitivity and specificity of using IDEIA compared to MT being 89% and 97%, respectively (PPV 92%; NPV 96%). Of the seven urine samples negative by IDEIA but positive by MT, the positive result was confirmed by the PCR or by tests on urethral samples for all but one of them. It is noteworthy that only 10 EBs or fewer were detected by MT in all seven IDEIA-negative urine samples, although the IDEIA was instrumental in finding positive 15 other urine samples which contained such a low number of EBs by MT. Of the five urine samples positive by IDEIA but negative by MT, two were not supported by other tests of either the urine or the urethra, and one of the two was obtained from a patient after antibiotic therapy had commenced.

Comparison of testing urethral smears by MT with urine deposits by IDEIA

Of 192 pairs of urethral and FPU specimens collected from 112 patients at their first and subsequent clinic visits, the results were in concordance for 176 of them (table 4), the sensitivity and specificity of using IDEIA for testing urine compared to MT for testing urethral smears being 82% and 95%, respectively (PPV 85%; NPV 94%). Nine patients had urethral smears positive by MT (supported by a positive PCR test in seven cases) but urines that were negative by IDEIA. Although five of these urines were positive by MT (two supported by the PCR), they contained 10 EBs or less, as did six of the MT-positive urethral smears. Of seven patients that had urines positive by IDEIA but urethral smears negative by MT, four had other tests on urine or the urethra that supported the positive findings.

Comparison of testing urethral smears by MT with urine deposits by any procedure

In this analysis it was assumed that a single positive result for a urine sample, whether it be by MT, PCR or IDEIA, was valid. Pairs of specimens (urethra, FPU) from 112 patients at their first clinic visit were tested. The results were in concordance for 96 of the pairs (table 5), the sensitivity and specificity of using any positive urine test compared to a urethral MT test being 92% and 81%, respectively. There were only four patients with a urethral smear positive by MT for whom *C trachomatis* was not found in urine by any test.

Comparison of testing urines before urethral swabbing with testing after swabbing

Since swabbing the urethra might influence the outcome of testing a urine sample, the result of testing urine from 34 patients before the urethral swab was compared with that of testing urine from 78 patients after the swab (table 6). It is clear, using MT or IDEIA, that the results for pre-swab and post-swab urines were similar.

Sensitivity of methods for detecting C trachomatis on first clinic visit

Urethral swabs and urines were available from 112 patients during their first clinic visit. Urethral swabs from 48 (43%) of them were positive by MT. *C trachomatis* was detected by MT in urine samples from 43 of these 48 men and also from five others

Table 3 Results of testing urine deposits by MT and by IDEIA

		No. of urines tested by IDEIA that were		
		Positive	*Negative*	*Total*
No. of urines tested by MT that were	Positive	57	7	64
	Negative	5	161	166
	Total	62	168	230

Table 4 Results of testing urethral smears by MT compared with urine deposits by IDEIA

		No. of urines tested by IDEIA that were		
		Positive	*Negative*	*Total*
No. of urethral smears tested by MT that were	Positive	40	9	49
	Negative	7	136	143
	Total	47	145	192

Table 5 Results of testing urethral smears by MT compared with urine deposits by any procedure

		No. of urines examined by any test* that were		
		Positive	*Negative*	*Total*
No. of urethral smears tested by MT that were	Positive	44	4	48
	Negative	12	52	64
	Total	56	56	112

*MT, PCR, or IDEIA

Table 6 Results of testing urines before urethral swabbing compared with those after swabbing

		No. of urine specimens taken							
		Before urethral swab that were				*After urethral swab that were*			
		Positive by		*Negative by*		*Positive by*		*Negative by*	
		MT	*IDEIA*	*MT*	*IDEIA*	*MT*	*IDEIA*	*MT*	*IDEIA*
No. of urethral smears that by MT were	Positive	12	11	1	2	31	29	4	6
	Negative	2	1	19	20	3	4	40	39
		92%	85%	90%	95%	89%	83%	93%	91%
		Sensitivity		Specificity		Sensitivity		Specificity	

whose urethral smears were negative (48 of 112; 43%). The sensitivity and specificity of detecting *C trachomatis* by MT in urine, compared to urethral swabs, were 90% and 92%, respectively. By comparison, IDEIA detected *C trachomatis* in urine samples from 39 patients who were positive in the urethra, and from an additional five (the results for four supported by other tests) who were negative in the urethra (44 of 112; 39%). Detection of *C trachomatis* in urine by IDEIA was 81% sensitive and 92% specific compared to its detection in the urethra by MT. When positive *C trachomatis* findings for urethral and urine samples by MT and IDEIA were combined, the overall detection rate in this group of men was 49% (55 of 112).

Discussion

A culture technique has been used previously to detect *C trachomatis* in urine samples from men but was not considered as sensitive as urethral swabbing.[7] However, the advent of other detection procedures offered the opportunity of re-appraising such a non-invasive approach and recent studies have suggested that it might have value.[8 9] We have had considerable experience of using the MicroTrak direct immunofluorescence technique to detect *C trachomatis* EBs[2 10] and in this study we used it as the "gold" standard. The PCR, which we found previously[3] to be as sensitive, but no more sensitive, than MT was used to support the MT technique. Testing a urine sample by MT to detect *C trachomatis* was not quite as sensitive as testing a urethral swab by MT. Since testing a urine sample by IDEIA was not as sensitive as testing a comparable sample by MT, it is not surprising that testing a urine sample by IDEIA was not as sensitive as testing a urethral swab by MT. However, the failure to detect organisms by IDEIA occurred in patients whose urine samples, or corresponding urethral swabs, contained few organisms as judged by MT. In addition, the division of such urine samples for testing by three techniques may have resulted in some aliquots containing insufficient organisms for detection. It would be logical to believe that the sensitivity could be enhanced by devoting the whole of a urine sample to examination by one procedure, such as IDEIA, rather than examining a portion only.

Swabbing the urethra before collecting a urine sample might either remove antigen available for detection in the urine, or by disruption of the epithelium increase the amount of antigen released in a subsequent urine specimen, leading to an overestimation of the sensitivity of detection in urine samples. Collecting the urine first might decrease the number of epithelial cells available for the urethral swab, thereby decreasing the sensitivity of the "gold" standard. However, the results of testing urine collected either before or after urethral swabbing revealed that there was no appreciable increase in the number of organisms detected by MT in a urine specimen collected after the urethral swab, although some sensitivity of the urethral specimen was lost by collecting urine first. Thus, we have no hesitation in saying that examination of urine, subsequent to a conventional urethral smear, by a sensitive procedure is an acceptable method of detecting *C trachomatis* in the male urethra. Others[8] have suggested that an EMU is superior to a FPU for detecting *C trachomatis*, but other observations of ours[11] have indicated that a FPU taken in the clinic is as least as valuable as an EMU.

We studied men with NGU because a large body of information on the prevalence of *C trachomatis* is available for them, up to 50% having been recorded in many studies.[1] In this study, 43% of the men were *C trachomatis*-positive as judged by a MT test on a urethral swab taken at the first clinic visit, the same proportion as for those whose chlamydial diagnosis was made on the basis of testing a urine sample. Furthermore, examination of urine enabled small as well as large numbers of EBs to be detected, particularly by MT, so that we see no reason why this non-invasive approach should not be applicable to male population groups, other than those with NGU, some of whom might be infected by few organisms.

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